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Single-axon action potentials in the rat hippocampal cortex

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Whether all action potentials propagate faithfully throughout axon arbors in the mammalian CNS has long been debated, and remains an important issue because many synapses occur far from the soma along extremely thin, unmyelinated, varicosity-laden branches of axon arbors. We detected unitary action potentials along individual axon branches of adult hippocampal CA3 pyramidal cells using extracellular electrodes, and analysed their conduction across long distances (mean, 2.1 mm) at 22 and 37 °C. Axons nearly always transmitted low-frequency impulses. At higher frequencies, most axons also transmitted impulses with striking fidelity. However, at paired-pulse frequencies in the hundreds of kilohertz range, axons exhibited variability: refractory periods ranged from 2.5 to 10 ms at 37 °C and from 5 to 40 ms at 22 °C. Although the basis for the refractory period variability could not be determined, these limits overlap with CA3 spike frequencies observed *in vivo*, raising the possibility that some axonal branches act as filters for the higher-order spikes in bursts, in contrast to the observed first-spike reliability. These results extend the observations of propagation reliability to a much longer distance and higher frequency domain than previously reported, and suggest a high safety factor for action potential propagation along thin, varicose axons.

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Neural communication relies on axonal propagation of regenerative sodium spikes for transmitting information over long distances. Impulse propagation along extremely thin, unmyelinated and densely varicose axons – exemplified by hippocampal CA3-to-CA1 (Schaffer collateral) axons – is understood mostly on the basis of electrophysiological population recordings (compound action potentials or prevolley; Andersen, 1960) or somatic invasion by antidromically conducted axonal spikes (Hessler *et al.* 1993; Allen & Stevens, 1994). More recently, studies using optical imaging of calcium transients as spike reporters have indicated high-fidelity axonal transmission (Frenguelli & Malinow, 1996; Mackenzie *et al.* 1996; Cox *et al.* 2000; Forti *et al.* 2000; Koester & Sakmann, 2000). However, optical methods are limited at high frequencies and over long distances. Moreover, both experimental and theoretical results indicate that spike failures can occur in unmyelinated axons under certain conditions (Krnjevic & Miledi, 1959; Lüscher & Shiner, 1990; Wall, 1995; Debanne *et al.* 1997).

We examined spike conduction reliability by recording action potentials from individual axons in distal parts of axon arbors. We chose to focus on CA3-to-CA1 branches in the arbors of CA3 cell axons, for several reasons. First, these are among the thinnest axons in the nervous system, with cores of just 1–4 microtubules and minimum diameters of only ~170 nm (Shepherd & Harris, 1998), yet have particularly abundant presynaptic varicosities (Shepherd *et al.* 2002; see also Fig. 1). These structural features imply biophysical limitations (e.g. high axial resistance), favouring propagation failure. Second, CA3 cells have elaborate and extensive axon arbors (Ishizuka *et al.* 1990; Li *et al.* 1994), and so are particularly well suited for single-axon recording from distal branches. Third, CA3 cells fire either at low frequencies or in high-frequency bursts ('complex spikes', Renshaw *et al.* 1940; Ranck, 1973), which may be important for information coding and learning (Lisman, 1997). Finally, the compound action potential of these axons has been characterised in some detail (Andersen, 1960), but remains incompletely understood because the underlying unitary action potentials have not been detected.

METHODS

Slices

Adult Wistar rats (1–3 months old) were deeply anaesthetised with ether (0.1–0.2 ml g^{-1}) and killed by decapitation, following institutional animal care guidelines. Both conventional transverse slices (300–400 μm thick) and longitudinal slices (Miles *et al.* 1988; Andersen *et al.* 2000) were used. Longitudinal slices were cut so as to contain large portions of the CA3–CA1 cortex and thus large portions of CA3 cell axon arbors. Longitudinally, these slices spanned at least two-thirds of the length of the hippocampus. Transversely, they extended from the mid-CA3 (at the level of the fimbria) to the mid-CA1 region. From the alvear surface, they had maximal thicknesses of 1 mm in the middle, where they contained the alveus and stratum oriens, the lucidum and a fraction of the radiatum. Slices were stored in a holding chamber at room temperature at the interface between the extracellular solution and humidified carbogen (5 % CO_2/95 % O_2). In the recording

chamber, slices were submerged and pinned on top of a mesh, to maximise exposure to the circulating solution. The extracellular solution contained (mM): NaCl 125, KCl 2.5, KH_2PO_4 1.25, $CaCl_2$ 2.5, $MgCl_2$ 1, $NaHCO_3$ 25 and glucose 16, and was bubbled with carbogen to maintain a pH of 7.4. Kynurenic acid (2 mM) or 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 10 μM) was added to the bath to block synaptic excitation. CNQX was purchased from Tocris (Ballwin, MO, USA) and kynurenic acid from Sigma (St Louis, MO, USA).

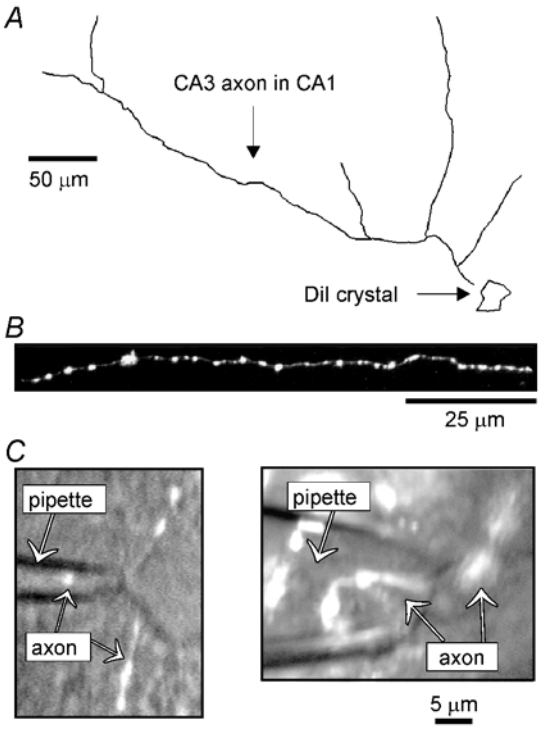

Figure 1. Morphological features of DiI-labelled CA3-to-CA1 axons, and the drawing up by negative pressure of a flexible loop of axon into a recording pipette

A, distal branches of CA3 axons travelling in area CA1 are often branched on a scale of hundreds of micrometres. A crystal of DiI was deposited in area CA1 radiatum of an acute slice in an interface chamber and incubated for several hours to label CA3-to-CA1 axons. The panel shows a tracing of the trajectory and branching pattern of a DiI-labelled axon. The number of branches formed by axons varied. Over distances of 1 cm or so (the greatest extent of labelling in these acute experiments), axons often branched several times, as seen in this example. Occasional axons had no identifiable branches over such distances. *B*, a confocal microscope image of a DiI-labelled axon showing that at higher magnification and resolution, CA3-to-CA1 axons are highly varicose. The straightness of this axonal branch on this scale is also typical for these axons. *C*, the same pressure parameters used for physiological single-axon recording caused individual DiI-labelled axons to be gently pulled into the recording pipette, as visualised in these examples using epifluorescence/differential interference contrast imaging. Varicosities along the axons are evident, but the axonal shaft segments between varicosities are often fainter due to their small diameter and the rapid bleaching of the dye that occurs during the procedure. The example on the left shows an axon that appears to enter and leave the pipette in an Ω form, whereas the one on the right may have been broken at one end. Some unlabelled axons presumably also entered the pipette together with the labelled one. These pictures demonstrate that axons could be sucked into pipettes with tip openings of 5 and 15 μm (left and right panel, respectively), but such visualisation was not used during the experiments in which electrical recordings were made.

Recording

Recording electrodes with tip diameters of 5–15 μm were fabricated from borosilicate, filled with extracellular solution and positioned in the stratum radiatum. Constant, gentle suction (−3 to −10 kPa) was applied, usually resulting in the detection of spontaneous unit activity for a transient period (5–60 s). Negative pipette pressure was necessary for the detection of single axonal units. Undoubtedly this caused many axons, not only the single axon being recorded from, to be pulled into the pipette. In separate experiments, we labelled CA3-to-CA1 axons with 1,1′-dioctadecyl-3,3,3′,3′-tetramethyl-indocarbocyanineperchlorate (DiI; Fig. 1*A* and *B*) and confirmed that suction pulled axons into the pipettes (Fig. 1*C*). The DiI-labelling methods are described in Shepherd *et al.* (2002). For a further description of the morphological features of CA3-to-CA1 axons, see Shepherd & Harris (1998) and references therein.

Signals were amplified with a DAM 50 amplifier (World Precision Instruments, Aston, UK) at × 1000 DC, low-pass filtered at 10 kHz, and digitised at 20 kHz. Response amplitudes were measured as the difference between the minimum and maximum in a 4 ms window, centred at the visually detected unit responses. This window width was set to include the observed latency jitter of clearly detectable units and the latency range observed at short stimulus intervals. Temperature was measured by a small probe close to the slice in the bath, and set at 22 ± 1 or 36 ± 1 °C. Two units were tested at 39 °C, but their parameters were not outside the distribution of the remaining population, and they were therefore included in the analysis.

Stimulation

The stimulation technique was based on the method of minimal stimulation (Raastad, 1995). Monopolar borosilicate electrodes (tip diameters 10–15 μm) were filled with extracellular solution. Single-axon responses were typically isolated as follows. To control for the general health of axons in slices we recorded population responses at short distances in the CA1 radiatum. The stimulating electrode was then moved elsewhere in the CA1 radiatum, 1–3 mm away. With this arrangement few axons would pass close to both electrode tips, since axons in the CA1 radiatum travel in a variety of directions (Shepherd *et al.* 2002) and many are cut by slicing. In addition, this distance was greater than distances traversed by dendrites or most non-Schaffer-type axons. These conditions thus greatly enhanced the likelihood of detecting single-axon spikes. We searched along ~200 μm vertical tracks, testing every 10–20 μm for a quantal increase in response amplitude above threshold. This was checked by first reducing the stimulus current to just below the threshold for a stimulus duration of ~100 μs, then presenting many stimuli with durations varying randomly from 50 to 150 μs (Fig. 2*D*). Here and throughout this study, threshold is defined as the minimum stimulus duration yielding reliable activation (mean, 81 ± 13 μs), rather than the minimum stimulus duration to give any activation (mean, 69 ± 11 μs). Only units with amplitudes well above the noise were studied. Mean single-axon response amplitudes (32–75 mV) averaged 3.6 times greater than the noise S.D. (never less than 2.2 times). In contrast, there were no significant differences between response amplitudes at low and high stimulation strength, as shown by dividing the stimulation range from threshold to maximal stimulus (150 μs) in half. The mean ratio of amplitudes in the strongest/weakest half-range was

0.99 ± 0.07 (S.D.; n = 21; not significantly different from 1.0). The combination of clear amplitude differences around threshold and the stability of amplitudes over a wide range of suprathreshold intensities makes it unlikely that the recordings were from more than a single unit. After recording single-axon responses, the stimulation intensity was increased to record compound action potentials at the same electrode positions to enable direct comparison of unitary and compound response latencies.

Unless indicated otherwise, data are presented as means ± S.D.

RESULTS

Recording from a single axon

We detected individual, small-amplitude, spike-like impulses in hippocampal area CA1 using extracellular recording. In this section we present evidence that these are individual action potentials travelling along single CA3-to-CA1 (Schaffer) axon branches, and describe some of their fundamental electrophysiological properties.

Spontaneous biphasic signals were detected during the application of negative pressure to recording pipettes placed in the stratum radiatum of area CA1 (Fig. 2*A*). Units recorded at any given site were often alike (Fig. 2*B*), suggesting that they arose from the same neural source. Similar units could be evoked by weak electrical stimulation at a distance in the same layer (Fig. 2*C*). Variations in stimulus strength around a threshold level ('minimal' stimulation; Raastad, 1995) revealed all-or-none impulses (Fig. 2*C*–*G*). Over a range of stimulation strengths, response amplitudes were constant (i.e. no additional units were recruited; see Methods). Occasionally two units with different latencies were activated (Fig. 2*E*). With much stronger stimulation, individual units merged into the compound action potential ('prevolley').

The latencies of these all-or-none spikes varied little for individual units. Figure 2*F* shows the variability (coefficient of variation, 1.5 %) for the unit in *C*–*E*. The average for all experiments was 3.6 ± 1.7 %. Interestingly, there was a weak dependence on stimulus intensity: the latency difference between the weakest (threshold) and strongest stimuli averaged 120 μs. We interpret that the

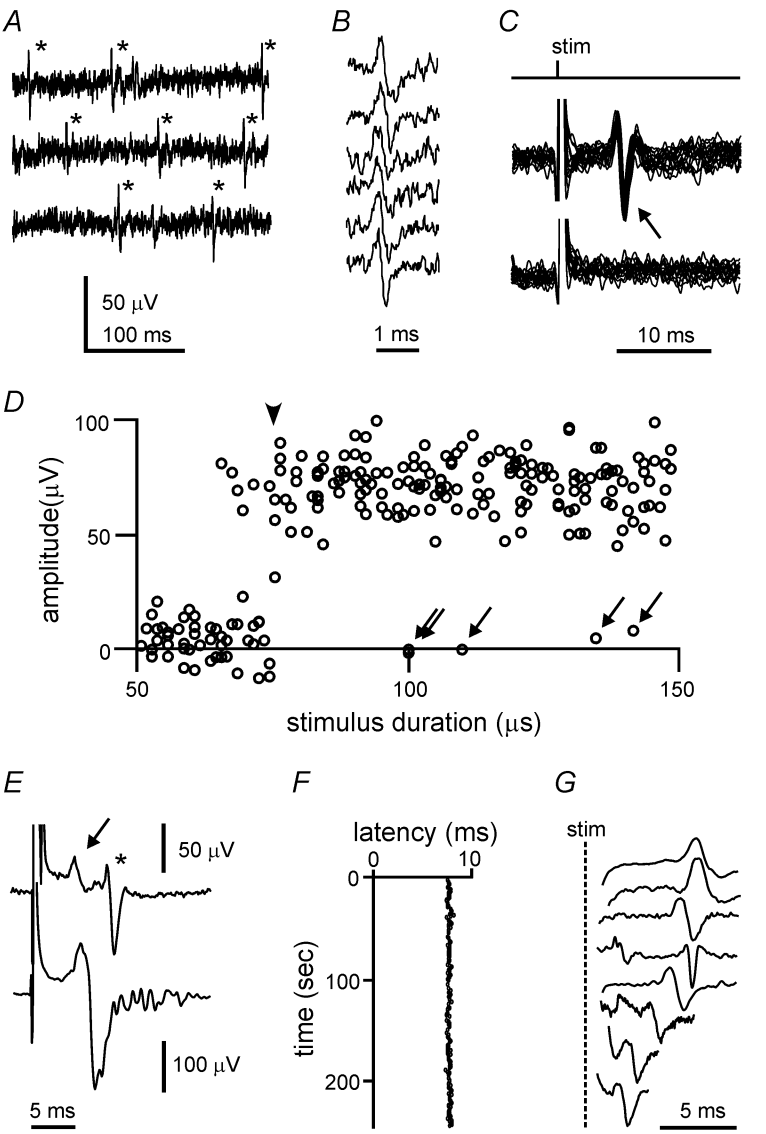

Figure 2. Single-axon action potentials

A, action potentials (asterisks) were recorded extracellularly from an axon in the CA1 stratum radiatum. *B*, spikes from *A* are shown aligned, and on a faster time scale. *C*, activation of single-axon action potentials (arrow) by minimal stimulation. Suprathreshold stimuli of varying intensities evoked spikes that varied little in waveform and latency (arrow, upper traces). Subthreshold stimuli did not evoke responses (lower traces). *D*, stimulus–response relationship of a single axon, showing that spike amplitude (peak-to-peak) was constant above the activation threshold (arrowhead) of ~75 μs. Five failures (arrows) out of 154 stimuli above threshold were not related to the stimulus strength. *E*, the upper trace shows activation of both the unit shown in *C* and *D* (asterisk) and a second, earlier unit (arrow) detected with slightly stronger stimuli (average of 30 traces). The response to stronger stimulation (lower trace) shows that single units had latencies within the time window of the population response. *F*, the latency of the unit from *C*–*E* was highly stable on a time scale of minutes. *G*, eight units from different experiments, normalised with respect to their peak-to-peak amplitude. stim, point at which the stimulus was given.

activation points moved slightly closer to the recording points by 24 μm on average (based on a conduction velocity of 0.20 m s^{-1} measured from population responses).

In contrast, latencies varied considerably for different units, but consistently fell within the time envelope of the compound response (Fig. 2*E*). We interpret the fluctuations at the tail of this compound response as units having particularly long conduction times, either because of longer path lengths or because of slower conduction velocities than the average axon. Similarly, waveforms for individual units were highly consistent, but varied among different units (Fig. 2*G*), at least partly due to the extracellular method of recording. These spikes were detected only when the stimulating and recording electrodes were both positioned in the CA3-to-CA1 pathway in the CA1 stratum radiatum, separated by long distances (mean 2.1 mm, range 1.2–2.9 mm). Although in principle some of these units might be from interneurons, this would be expected to occur only rarely, since inhibitory axons are greatly outnumbered by CA3-to-CA1 axons in the neuropil of the CA1 radiatum (Hjorth-Simonsen, 1973; Shepherd & Harris, 1998), and the axon arbors of many interneurons project much more locally than those of CA3 cells. Moreover, as shown below, unitary responses closely resembled compound CA3-to-CA1 responses. We therefore conclude that these spikes represent single action potentials travelling along individual branches of Schaffer-type axons.

Conduction is reliable at low frequency

Since pyramidal neurons in the hippocampus often fire at low rates *in vivo* (Ranck, 1973), we investigated the conduction reliability of axons in this frequency regime. A striking property of most of these axons was that response failures were rare at low stimulation frequency (1 Hz). For example, the failure rate of the unit shown in Fig. 2*D* was 3 % (5 failures per 154 suprathreshold stimulations). We studied single-axon action potentials in 13 experiments at 22 °C and in eight experiments at 37 °C (15 axonal units) and observed only 148 failures with 4236 suprathreshold stimuli – an overall failure rate of 3.5 % (Fig. 3*A*). Two units accounted for the majority of failures, failing at rates of 55 % and 25 %. The median success rate at both temperatures was 100 %. There was no significant difference in failure rate at the two temperatures (Fig. 3*B*, Kolmogorov-Smirnov test, $P > 0.1$), but because failures were rare, small effects of temperature on their rates would be difficult to detect. The results suggest, however, that the temperature did not substantially affect failure rates.

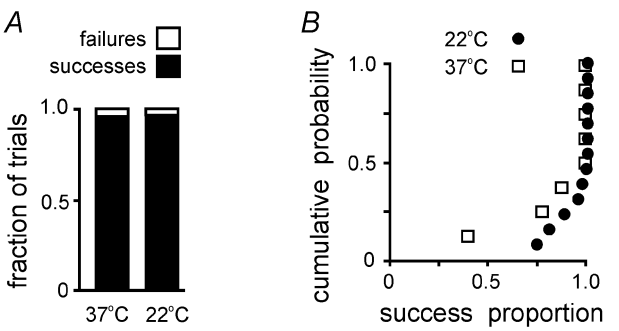

Figure 3. The proportion of failures was low

A, the total number of failures and successes, normalised, at high and low temperature. *B*, a cumulative plot of the success proportion for eight units at 22 °C and 13 units at 37 °C.

Conduction properties are modulated at short intervals

As hippocampal pyramidal neurons also fire in short, rapid bursts *in vivo* (Renshaw *et al.* 1940; Ranck, 1973), we also wanted to test the ability of these axons to conduct action potentials reliably at higher frequencies. We did so with a paired-pulse protocol, randomly varying inter-stimulus intervals from 1 to 40 ms for a pair of stimuli presented at 1 Hz. Rapid stimulus repetition resulted in changes in the amplitude, latency and failure rate of the second response.

A representative example for a unit tested at both physiological and room temperature is shown in Fig. 4. At all paired-pulse intervals greater than 20 ms (and with suprathreshold stimulus strengths) the spike always conducted (asterisks in Fig. 4*A*). Thresholds were lower at physiological than at room temperature (Fig. 4*B*, left *vs.* right panels), but similar for the first and second responses (Fig. 4*B*, upper *vs.* lower panels). The threshold interval (the shortest interval giving two spikes) was 5 ms at 37 °C and 10 ms at 22 °C (Fig. 4*C* and *D*). Second spikes arrived slightly later and with lower amplitudes for intervals just above the threshold interval (Fig. 4*C* and *D*).

Do threshold intervals measured in this way represent absolute refractory periods? We tested this by approximately doubling the stimulus strength; specifically, it was set to the maximum value of 150 μs, an increase over threshold of 205 % on average and at least 150 % for each axon. Axons remained refractory to these strong stimuli, indicating a steep increase in threshold at the minimum effective paired-pulse intervals, probably reflecting absolute refractory periods. The part of the axon between the recording and stimulation point that failed to respond could not be determined with this method.

Average values for the threshold interval, amplitude reduction and latency increase are shown in Fig. 5, including eight units at physiological temperature and 13 at room temperature. The average threshold interval was 6.3 ± 1.7 ms at high and 11.5 ± 2.6 ms at low temperature, the difference being significant ($P < 0.03$, Kolmogorov-Smirnov test). The average amplitude ratio (second:first) was 0.65 ± 0.05 at high and 0.76 ± 0.03 at low temperature, and the average latency ratio (second:first) was 1.07 ± 0.01 at high and 1.07 ± 0.02 at low temperature.

Comparison of unitary and compound action potentials

Population recordings effectively average across large numbers of axons, providing a way to confirm and to investigate further the conduction properties and modulations found at the single-axon level. We therefore measured compound action potentials of CA3-to-CA1 axons evoked by the same paired-pulse protocol used on single axons. The top trace in Fig. 6*A* shows that the second response was smaller at a repetition interval of 7 ms. Testing many intervals from 1 to 40 ms at two temperatures gave the families of responses shown in Fig. 6*A*. Shortening the stimulus interval caused progressive amplitude reduction, leading to refractoriness.

Similar results were obtained for 10 experiments, including five tested orthodromically (stimulating near CA3) and five tested antidromically (stimulating near the subiculum). Average values for the second:first amplitude ratio, shown for high and low temperature in Fig. 6*B*, indicate a strong interval dependence of the amplitude of the compound action potential. In addition, there were no detectable responses at latencies of less than 2 ms at high temperature and less than 4 ms at low temperature, indicating that only a minute fraction of the axons could have been activated by the second but not the first pulse in these experiments.

What mechanisms underlie the frequency dependence of the population response? Two lines of evidence suggest that both refractoriness and amplitude modulation at the single-axon level play a role. First, the amplitude reduction observed at the single-axon level (Figs 4*D* and 5*B*) was substantial, particularly at physiological temperature. Second, the refractory periods measured for single axons corresponded closely to the period when the compound

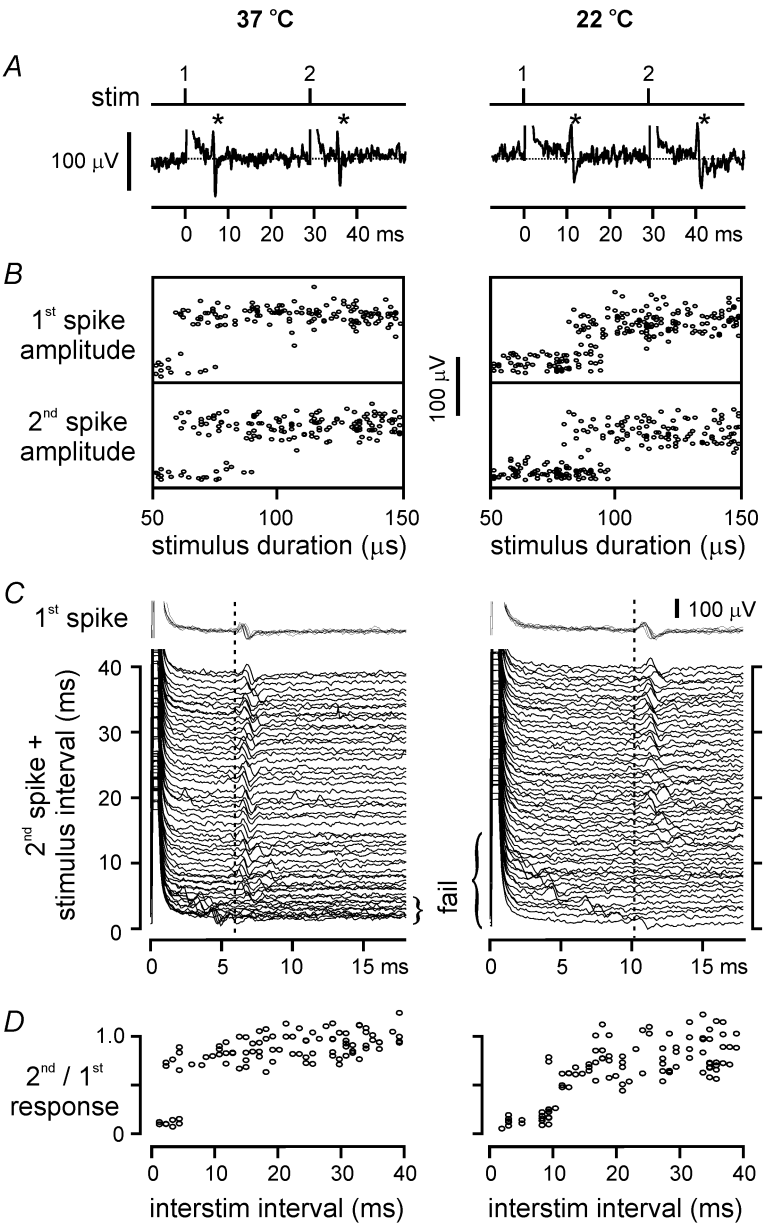

Figure 4. Analysis of the shortest response interval for one axon at two temperatures

A, action potentials (asterisks) were evoked by paired stimuli (same intensity, 27 ms interval). *B*, first- and second-spike amplitudes as a function of stimulus intensity. *C*, responses to paired suprathreshold stimuli with interstimulus intervals of 1–40 ms. The top traces are superimposed responses to the first stimulus in each pair. The bottom traces are responses to the second stimulus in each pair, offset vertically for display according to the pair's interstimulus intervals (randomly ordered during the experiment). For the shortest intervals, no second responses occurred (marked 'fail'). Traces at these short intervals did contain first responses, which appeared because of the short interstimulus intervals. *D*, ratios of second/first response amplitudes, as a function of the interstimulus interval.

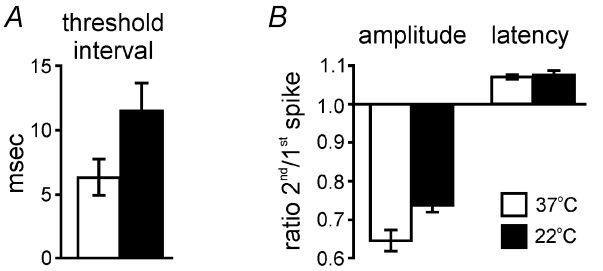

Figure 5. Average results at high temperature and low temperature

A, the minimum (threshold) intervals for eliciting a second response. *B*, the amplitude and latency of the second response, relative to the first.

response amplitude was most affected. The need for two processes to explain the amplitude reduction of the population response was also supported by the fact that two exponential functions were needed to fit the data (continuous lines in Fig. 6*B*).

The fast exponential components (continuous lines in Fig. 6*C*) were not significantly different from the cumulative distributions of the threshold intervals from the single-axon experiments (circles; Kolmogorov-Smirnov test) at either temperature, making it likely that the two data sets describe the same process, namely the distribution of absolute refractory periods. We conclude that refractoriness influences responses in this time window, and refractory periods vary considerably between axons, as described by the exponential functions. Furthermore, the similarity between population and unitary data provides further evidence that they represent the same population of axons.

DISCUSSION

Reliability of single-axon action potentials

Schaffer-type axons, despite being unmyelinated, densely varicose, branching and among the thinnest axons in the mammalian CNS, apparently conduct low-frequency action potentials as reliably as thick, myelinated axons. At a stimulation frequency of 1 Hz, typical for CA3 cell firing rates *in vivo*, almost no failures were observed. We reached this conclusion by directly recording voltage signals from distal axon branches, supporting previous evidence obtained from calcium imaging of more proximal branches (Cox *et al.* 2000; Forti *et al.* 2000; Koester & Sakmann, 2000) and CA3 soma recordings (Hessler *et al.* 1993; Allen & Stevens, 1994). Electrical recordings from individual cerebellar parallel fibres (Merrill *et al.* 1978),

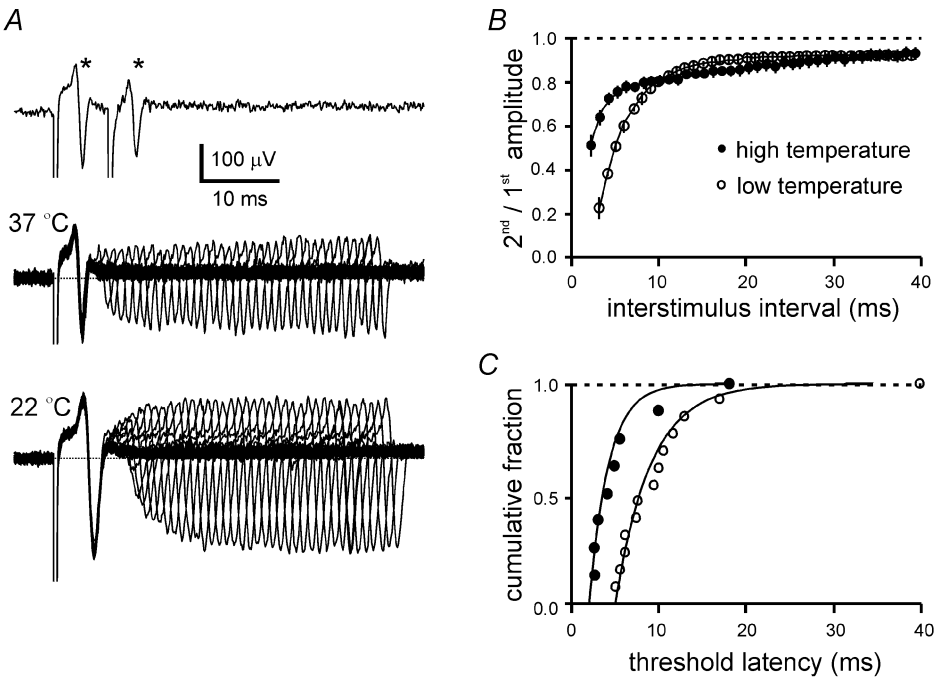

Figure 6. Comparison of compound and unitary action potential refractory periods

A, upper trace, typical compound action potentials (asterisks) from a population of CA3-to-CA1 axons, elicited with two identical stimuli separated by 7 ms. Lower traces, responses at two temperatures evoked by paired pulses separated by intervals of 1–40 ms (presented in random order). Second-stimulus artefacts have been removed. *B*, peak-to-peak amplitude of the second response, normalised to the first, as a function of the interstimulus interval. Lines represent double-exponential fits to the data. *C*, the fast exponential functions measured from populations of axons (continuous lines) closely matched the distributions of refractory periods measured from single axons (circles).

which morphologically resemble CA3 axons, also indicated high reliability at low frequencies.

Pyramidal CA3 cells can also fire high-frequency bursts of action potentials with only a few milliseconds between spikes (Ranck, 1973). We found that many axons were also highly reliable at higher stimulus frequencies. Some axons were able to fire two action potentials with intervals < 2 ms at 37 °C (i.e. they matched the maximal frequencies recorded *in vivo* from the somata of these cells). Thus a second conclusion of this study is that at least some axons appear capable of faithfully propagating, over long distances, all of the spikes in a physiological burst.

At the shortest effective repetition intervals, spike amplitudes were reduced by 25–35 %. Although slower conduction would contribute to the amplitude reduction, we observed only a small (7 %) decrease in velocity. The observed amplitude reduction thus suggests a substantial safety factor for axonal action potential propagation (i.e. approximately 25 % more sodium current available than is strictly needed for low-frequency conduction). Supporting this are the observations of a high safety factor for axonal conduction in cultured hippocampal neurons (Mackenzie & Murphy, 1998), and for somatic spike generation in acutely dissociated hippocampal neurons, where action potentials only fail when at least 30–50 % of the somatic sodium current is blocked (Madeja, 2000). Interestingly, sodium channel inactivation caused by high-frequency spiking could affect synaptic transmission; for example, in cultured hippocampal neurons it has been shown to contribute to short-term depression during brief spike trains (Brody & Yue, 2000; He *et al.* 2002).

Do some axons filter high-frequency bursts of action potentials?

From *in vivo* experiments on rats, cats and rabbits it is known that CA3 commissural fibre populations in the CA1 stratum radiatum are refractory to extracellular activation at intervals of < 2.5 ms (Andersen, 1960). We found similar minimal intervals, but in addition considerably longer refractory periods for some fibres, a finding that would not have been possible without single-axon recordings. Including these longer intervals, the mean refractory period was 6.3 ms at 34 °C, longer than many intervals recorded from CA3 somata *in vivo* (Harris *et al.* 2001).

Single-axon recording enables the measurement of axonal refractoriness (to extracellular activation) at the level of individual axonal branches, but does not reveal whether refractoriness occurs (1) at the stimulation site, (2) at branch points or other sites along the axon, or (3) along the entire axon. Localising the sites of high-frequency failures will ultimately depend on the development of imaging techniques with high temporal and spatial resolution. With regard to these different possibilities, it noteworthy that local refractoriness at the stimulation site, arising, for example, from extracellular potassium accumulation (due to the synchronous activation of many axons and dendrites), has important implications for experiments involving the extracellular activation of axons. This is particularly relevant since refractory periods were longest at room temperature, a condition commonly used in studies of CA3-to-CA1 synapses. With an interstimulus interval of 10 ms, about 20 % of axons failed in response to the second stimulus (Fig. 6*C*).

If different axons or branches do have different absolute refractory periods (possibilities (2) or (3) above), this could be important for synaptic transmission. These effects could be heterogeneous at different synapses, even along the same axon, due to variability in synaptic release properties. In particular, facilitation at CA3-to-CA1 synapses would tend to enhance the differential effects of action potential successes and failures. Many CA3-to-CA1 synapses release transmitter only in response to the second spike in a burst (Stevens & Wang, 1995; Lisman, 1997; Hanse & Gustafsson, 2001), leaving large controlling power to the frequency-following capacity of the axon.

Thus, at high firing rates, frequency-dependent axonal spike conduction, in combination with short-term synaptic plasticity, could extend the repertoire of signalling mechanisms occurring in neural circuits. Alternatively, should thin, varicose, axons remote from the soma prove to be even more reliable frequency followers than observed here, even greater importance would be accorded to the precise timing of spikes in a burst in combination with the precise frequency dependence of the sign and magnitude of short-term plasticity mechanisms modulating transmission at individual synapses along the varicose axon.

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